

NEB-207-US.ST25.txt SEQUENCE LISTING

Morgan, Richard D. <110> Bhatia, Tanya Davis, Theodore Lovasco, Lindsay Recombinant Type II Restriction Endonucleases, MmeI And Other <120> Related Endonucleases And Methods For Producing The Same <130> NEB-207-US <150> us 60/395,431 2002-07-12 <151> <160> 50 <170> PatentIn version 3.2 <210> <211> 6010 <212> DNA Methylophilus methylotrophus <213> <220> misc_feature <221> <222> (800)..(800)<223> n is a, c, g, or t <400> gaattccaga taggtagtcc tttggtactt ccatcccaac cagtgtcacg ttccgcgcca 60 aaccaatcgg ttaaagtgta agaaagtctt gcactgaagt agctgtagga caaaccgaag 120 ttaacctctg tggtatccca gcgaccacct ttaggtgttt gacggaagcc tgctgcgtca 180 cctgccaagt tatatttctt ccatgaacca cctgggtaca ggtagctgat caaaccagca 240 gtccaaccca agccttcaat agcaggaata gttccgttat acccaccata aatatcaatt 300 tcggcagttg catcagggaa ggtatttggt gtcacgtttg aaccccatgc accgacataa 360 aagccgctgt catgagtaat atcaataccg ccttgaacgg caggtttgtg ccagttttgt 420 480 gaaataccac gagcatagta atctgaaaca aatccaacgt ttgcagtagc agcccaggct gatttttctt ctttagcctc ttcagctgcg tatgaaactt gggcaaaaga taatgtgctt 540 aacactgctg tgagcaatat agattgacgc attatgagtc ctctctctgt gaaatctttg 600 attaagttgt tgtaaacgag aatgaaacaa caaccacaaa gcaaagcacg tgccaaacta 660 720

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545

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Leu Leu Lys Ala His Gln Lys Leu Asp Lys Ala Val Asp Ser Val Tyr 865 870 875 880

Gly Phe Lys Gly Pro Asn Thr Glu Ile Ala Arg Ile Ala Phe Leu Phe 885 890 895

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Ser Ser Asn Ile Ser His Cys Lys Asn Ile Asn Gly Tyr Leu Phe Asp 50 55 60

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Pro Arg Met His Asn Gly Cys Lys Leu Leu Asp Gly Gly Phe Tyr Thr 85 90 95

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Leu Phe Gln Glu Tyr Ile Glu Thr Lys Thr Leu Glu Asp Gly Ser Asp 210 215 220 Leu Ala His His Ile Asn Thr Leu Phe Tyr Val Leu Asn Thr Pro Glu 225 230 235 240 Gln Lys Arg Leu Lys Asn Leu Asp Glu His Leu Ala Ala Phe Pro Tyr 245 250 255 Ile Asn Gly Lys Leu Phe Glu Glu Pro Leu Pro Pro Ala Gln Phe Asp 260 265 270 Lys Ala Met Arg Glu Ala Leu Leu Asp Leu Cys Ser Leu Asp Trp Ser Ile Ser Pro Ala Ile Phe Gly Ser Leu Phe Gln Ser Ile Met Asp 290 295 300 Ala Lys Lys Arg Arg Asn Leu Gly Ala His Tyr Thr Ser Glu Ala Asn 305 310 315 320 Ile Leu Lys Leu Ile Lys Pro Leu Phe Leu Asp Glu Leu Trp Val Glu 325 330 335 Phe Glu Lys Val Lys Asn Asn Lys Asn Lys Leu Leu Ala Phe His Lys Lys Leu Arg Gly Leu Thr Phe Phe Asp Pro Ala Cys Gly Cys Gly Asn 355 360 365 Phe Leu Val Ile Thr Tyr Arg Glu Leu Arg Leu Leu Glu Ile Glu Val 370 375 380 Leu Arg Gly Leu His Arg Gly Gly Gln Gln Val Leu Asp Ile Glu His 385 390 395 400 400 Leu Ile Gln Ile Asn Val Asp Gln Phe Phe Gly Ile Glu Ile Glu Glu Phe Pro Ala Gln Ile Ala Gln Val Ala Leu Trp Leu Thr Asp His Gln 420 430 Met Asn Met Lys Ile Ser Asp Glu Phe Gly Asn Tyr Phe Ala Arg Ile Pro Leu Lys Ser Thr Pro His Ile Leu Asn Ala Asn Ala Leu Gln Ile Page 15

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705

Ser Ser Ala Lys Pro Thr Arg Gln Ser Ala Ser Thr Pro Met Lys Phe 725 730 735

Phe Tyr Ile Ser Gln Pro Asp Thr Asp Tyr Leu Leu Ile Pro Glu Thr 740 745 750

Ser Ser Glu Asn Arg Gln Phe Ile Pro Ile Gly Phe Val Asp Arg Asn 755 760 765

Val Ile Ser Ser Asn Ala Thr Tyr His Ile Pro Ser Ala Glu Pro Leu 770 780

Ile Phe Gly Leu Leu Ser Ser Thr Met His Asn Cys Trp Met Arg Asn 785 790 795 800

Val Gly Gly Arg Leu Glu Ser Arg Tyr Arg Tyr Ser Ala Ser Leu Val 805 810 815

Tyr Asn Thr Phe Pro Trp Ile Gln Pro Asn Glu Lys Gln Ser Lys Ala 820 825 830

Ile Glu Glu Ala Ala Phe Ala Ile Leu Lys Ala Arg Ser Asn Tyr Pro 835 840 845

Asn Glu Ser Leu Ala Gly Leu Tyr Asp Pro Lys Thr Met Pro Ser Glu 850 855 860

Leu Leu Lys Ala His Gln Lys Leu Asp Lys Ala Val Asp Ser Val Tyr 865 870 875 880

Gly Phe Lys Gly Pro Asn Thr Glu Ile Ala Arg Ile Ala Phe Leu Phe 885 890 895

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Glu Phe Phe Ala Ile Phe Gly Ile Asp Arg Lys Asn Val Ala His Phe 35 40 45

Glu Tyr Pro Val Lys Asp Pro Ala Asp Asn Thr Gln Phe Val Asp Ile
50 60

Phe Trp Glu Gly Ile Phe Leu Ala Glu His Lys Ser Ala Asn Lys Asn 65 70 75 80

Leu Thr Lys Ala Lys Glu Gln Ala Glu Arg Tyr Leu Gln Glu Ile Gly 85 90 95

Arg Thr Lys Pro Ser Ala Leu Pro Glu Tyr Tyr Ala Val Ser Asp Phe $100 \hspace{1cm} 105 \hspace{1cm} 110$

Ala His Phe His Leu Tyr Arg Arg Val Pro Glu Glu Gly Ala Glu Asn 115 120 125

Gln Trp Gln Phe Pro Leu Glu Glu Leu Pro Glu Tyr Ile Thr Arg Gly 130 135 140

Val Phe Asp Phe Met Phe Gly Ile Glu Ala Lys Val Arg Gln Ile Gln 145 150 155 160

Glu Glu Ala Asn Ile Gln Ala Ala Thr Ile Gly Arg Leu His Asp 165 170 175

Ala Leu Lys Glu Glu Gly Ile Tyr Glu Glu His Glu Leu Arg Leu Phe 180 185 190

Ile Thr Arg Leu Leu Phe Leu Phe Phe Ala Asp Asp Ser Ala Val Phe 195 200 205

Arg Arg Asn Tyr Leu Phe Gln Asp Phe Leu Glu Asn Cys Lys Glu Ala 210 215 220

Asp Thr Leu Gly Asp Lys Leu Asn Gln Leu Phe Glu Phe Leu Asn Thr 225 230 235 240

Pro Asp Gln Lys Arg Ser Lys Thr Gln Ser Glu Lys Phe Lys Gly Phe Page 18

Glu Tyr Val Asn Gly Gly Leu Phe Lys Glu Arg Leu Arg Thr Phe Asp 260 265 270 Phe Thr Ala Lys Gln His Arg Ala Leu Ile Asp Cys Gly Asn Phe Asp 275 280 285 Trp Arg Asn Ile Ser Pro Glu Ile Phe Gly Thr Leu Phe Gln Ser Val 290 295 300 Met Asp Ala Gln Glu Arg Arg Glu Ala Gly Ala His Tyr Thr Glu Ala 305 310 315 320 Ala Asn Ile Asp Lys Val Ile Asn Gly Leu Phe Leu Glu Asn Leu Arg 325 330 335 Ala Glu Phe Glu Ala Val Lys Ala Leu Lys Arg Asp Lys Ala Lys Lys 340 345 350 Leu Ala Ala Phe Tyr Gln Lys Ile Gln Asn Leu Gln Phe Leu Asp Pro 355 360 365 Ala Cys Gly Cys Gly Asn Phe Leu Ile Val Ala Tyr Asp Arg Ile Arg 370 380 Ala Leu Glu Asp Asp Ile Ile Ala Glu Ala Leu Lys Asp Lys Ala Asp 385 390 395 400 Gly Leu Phe Asp Ser Pro Ser Val Gln Cys Arg Leu Lys Gln Phe His 405 410 415 Gly Ile Glu Ile Asp Glu Phe Ala Val Leu Ile Ala Arg Thr Ala Met 420 425 430 Trp Leu Lys Asn His Gln Cys Asn Ile Arg Thr Gln Ile Arg Phe Asp 435 440 445 Gly Glu Val Ala Cys His Thr Leu Pro Leu Glu Asp Ala Ala Glu Ile 450 455 460 Ile His Ala Asn Ser Leu Arg Thr Pro Trp Gln Ala Ala Asp Tyr Ile Phe Gly Asn Pro Pro Phe Ile Gly Ser Thr Tyr Gln Thr Lys Glu Gln 490

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Gly Asn Tyr Leu Ile Ile Pro Ser Val Ser Ser Glu Ser Arg Arg Phe 755 760 765

Ile Pro Ile Gly Tyr Leu Ser Phe Glu Thr Val Val Ser Asn Leu Ala 770 780

Phe Ile Leu Pro Asn Ala Thr Leu Tyr His Phe Gly Ile Leu Ser Ser 785 790 795 800

Thr Met His Asn Ala Phe Met Arg Thr Val Ala Gly Arg Leu Lys Ser 805 810 815

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Glu Ser Cys Arg Leu Pro Ser Glu Asn Asp Arg Pro Asp Pro Leu Arg 835 840 845

Ala Ala Val Glu Ala Ala Ala Gln Thr Val Leu Asp Ala Arg Gly Gln 850 860

Tyr Arg Arg Glu Ala Gln Glu Ala Gly Leu Pro Glu Pro Thr Leu Ala 865 870 875 . 880

Glu Leu Tyr Ala Pro Asp Ala Gly Tyr Thr Ala Leu Asp Lys Ala His 885 890 895

Ala Thr Leu Asp Lys Ala Val Asp Lys Ala Tyr Gly Tyr Lys Thr Gly 900 905 910

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Pro Asp Ile Phe Gly Ser Met Ile Gln Ala Val Ala Ser Glu Glu Ser 275 280 285 Ser Tyr Leu Gly Met His Tyr Thr Ser Val Pro Asn Ile Met Lys 290 295 300 Val Ile Lys Pro Leu Phe Leu Asp Lys Leu Asn Gln Ser Phe Leu Asp 305 310 315 320 Ala Tyr Asp Asp Tyr Thr Lys Leu Glu Asn Leu Leu Thr Arg Ile Gly 325 330 335 Lys Ile Lys Phe Phe Asp Pro Ala Cys Gly Ser Gly Asn Phe Leu Ile 340 345 350 Ile Thr Tyr Lys Glu Leu Arg Arg Met Glu Ile Asn Ile Ile Lys Arg 355 360 365 Gln Glu Leu Leu Gly Glu Tyr Leu Tyr Val Pro Ser Val Thr Leu 370 380 Ser Gln Phe Tyr Gly Ile Glu Ile Glu Asp Phe Ala His Asp Val Ala 385 390 395 400 Lys Leu Ser Leu Trp Ile Ala Glu His Gln Met Asn Glu Glu Leu Lys 405 410 415 Asn Glu Val His Asn Ala Val Arg Pro Thr Leu Pro Leu His Thr Ala 420 425 430 Gly Asp Ile Arg Cys Ala Asn Ala Ile Arg Val Glu Trp Thr Glu Val 435 440 445 Cys Pro Ala Gln Gly Ser Glu Glu Val Tyr Val Phe Gly Asn Pro Pro 450 460 Tyr Leu Gly Ser Lys Lys Gln Asn Lys Glu His Lys Ser Asp Met Leu 465 470 475 480 Ser Ile Phe Gly Lys Val Lys Asn Gly Lys Met Leu Asp Tyr Ile Ser 485 490 495 Ala Trp Phe Tyr Phe Gly Ala Lys Tyr Ala Ser Thr Thr Asn Ala Lys 500 510 Val Ala Phe Val Ser Thr Asn Ser Val Thr Gln Gly Glu Gln Val Ser 515 520 525 Page 23

Ile Leu Trp Asn Glu Leu Phe Lys Phe Gly Ile Gln Ile Asn Phe Ala 530 540 Tyr Lys Ser Phe Lys Trp Ala Asn Asn Ala Lys Asn Asn Ala Ala Val 545 550 555 560 Ile Val Val Ile Val Gly Phe Gly Pro Leu Asp Thr Lys Val Asn Lys 565 570 575 Tyr Leu Phe Val Asp Glu Thr Lys Lys Leu Val Ser Asn Ile Ser Pro 580 585 590 Tyr Leu Thr Asp Gly Glu Asn Ile Leu Val Ser Ser Arg Thr Lys Pro 595 600 605 Ile Ser Asp Leu Pro Lys Leu His Phe Gly Asn Met Pro Asn Asp Gly 610 620 Gly Gly Leu Leu Phe Thr Ile Thr Glu Tyr Thr Asp Ala Ile Asn Lys 625 630 635 Tyr Pro Glu Leu Val Pro Tyr Phe Lys Lys Phe Ile Gly Ser Val Glu 645 650 655 Phe Ile Asn Gly Gly Leu Arg Tyr Cys Leu Trp Leu Asn Glu Ala Lys 660 670 Tyr Glu Lys Ile Lys Ser Asn Pro Leu Ile Gln Glu Arg Ile Ser Ile 675 680 685 Ser Lys Asn His Arg Glu Lys Ser Thr Asp Lys Gly Thr Asn Lys Leu 690 700 Ala Leu Thr Pro Trp Lys Phe Arg Asp Thr His Glu Thr Thr Asn Tyr 705 710 715 720 Ser Ile Val Val Pro Ser Val Ser Ser Glu Asn Arg Phe Tyr Ile Pro 725 730 735 Met Gly Leu Ala Gly Ala Asp Thr Ile Leu Ser Asn Leu Ile Tyr Val 740 745 750 Ile Tyr Asp Ala Glu Ile Tyr Leu Leu Gly Ile Leu Met Ser Arg Met 755 760 765 His Met Thr Trp Val Lys Ala Val Ala Gly Arg Leu Lys Thr Asp Tyr

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Arg Tyr Ser Ala Gly Leu Cys Tyr Asn Thr Phe Pro Ile Pro Glu Leu 785 790 795 800

Ser Thr Arg Arg Lys Asn Glu Ile Glu Glu Ala Ile Leu Glu Ile Leu 805 810 815

Asp Leu Arg Glu Glu Gln Gly Gly Thr Leu Ala Glu Leu Tyr Asn Pro 820 825 830

Ser Thr Met Pro Ile Glu Leu Lys Val Ala His Glu Lys Leu Asp Gly 835 840 845

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Trp Gly Asp Leu Leu Asp Cys Phe Gly Val Asn Ala Arg Asp Leu Tyr 50 60

Leu Tyr Gln Arg Ser Ala Lys Arg Ala Ser Thr Gly Arg Thr Gly Lys 65 70 75 80

Ile Asp Met Phe Met Pro Gly Lys Val Ile Gly Glu Ala Lys Ser Leu 85 90 95

Gly Val Pro Leu Asp Asp Ala Tyr Ala Gln Ala Leu Asp Tyr Leu Leu 100 105 110 Page 25

Gly Gly Thr Ile Ala Asn Ser His Met Pro Ala Tyr Val Val Cys Ser 115 120 125 Phe Glu Thr Leu Arg Val Thr Arg Leu Asn Arg Thr Tyr Val Gly 130 140 Asp Ser Ala Asp Trp Asp Ile Thr Phe Pro Leu Ala Glu Ile Asp Glu 145 150 155 160 His Ile Glu Gln Leu Ala Phe Leu Ala Asp Tyr Glu Thr Ser Ala Tyr 165 170 175 Arg Glu Glu Glu Lys Ala Ser Leu Glu Ala Ser Arg Leu Met Val Glu Leu Phe Arg Ala Met Asn Gly Asp Asp Val Asp Glu Ala Val Gly Asp 195 200 205 Ala Pro Thr Thr Pro Glu Glu Glu Asp Glu Arg Val Met Arg Thr 210 215 220 Ser Ile Tyr Leu Thr Arg Ile Leu Phe Leu Leu Phe Gly Asp Asp Ala 225 230 235 240 Gly Leu Trp Asp Thr Pro His Leu Phe Ala Asp Phe Val Arg Asn Glu 245 250 255 Thr Thr Pro Glu Ser Leu Gly Pro Gln Leu Asn Glu Leu Phe Ser Val 260 265 270 Leu Asn Thr Ala Pro Glu Lys Arg Pro Lys Arg Leu Pro Ser Thr Leu 275 280 285 Ala Lys Phe Pro Tyr Val Asn Gly Ala Leu Phe Ala Glu Pro Leu Ala 290 295 300 Ser Glu Tyr Phe Asp Tyr Gln Met Arg Glu Ala Leu Leu Ala Ala Cys 305 310 315 320 Asp Phe Asp Trp Ser Thr Ile Asp Val Ser Val Phe Gly Ser Leu Phe 325 330 335 Gln Leu Val Lys Ser Lys Glu Ala Arg Arg Ser Asp Gly Glu His Tyr 340 350 Thr Ser Lys Ala Asn Ile Met Lys Thr Ile Gly Pro Leu Phe Leu Asp Page 26

Glu Leu Arg Ala Glu Ala Asp Lys Leu Val Ser Ser Pro Ser Thr Ser 370 380 Val Ala Ala Leu Glu Arg Phe Arg Asp Ser Leu Ser Glu Leu Val Phe 385 390 395 400 Ala Asp Met Ala Cys Gly Ser Gly Asn Phe Leu Leu Leu Ala Tyr Arg 405 410 415 Glu Leu Arg Arg Ile Glu Thr Asp Ile Ile Val Ala Ile Arg Gln Arg 420 425 430 Arg Gly Glu Thr Gly Met Ser Leu Asn Ile Glu Trp Glu Gln Lys Leu 435 440 445 Ile Gly Gln Phe Tyr Gly Ile Glu Leu Asn Trp Trp Pro Ala Lys 450 460 Ile Ala Glu Thr Ala Met Phe Leu Val Asp His Gln Ala Asn Lys Glu 465 470 475 480 Leu Ala Asn Ala Val Gly Arg Pro Pro Glu Arg Leu Pro Ile Lys Ile 485 490 495 Thr Ala His Ile Val His Gly Asn Ala Leu Gln Leu Asp Trp Ala Asp 500 505 510 Ile Leu Ser Ala Ser Ala Ala Lys Thr Tyr Ile Phe Gly Asn Pro Pro 515 520 525 Phe Leu Gly His Ala Thr Arg Thr Ala Glu Gln Ala Gln Glu Leu Arg 530 540 Asp Leu Trp Gly Thr Lys Asp Ile Ser Arg Leu Asp Tyr Val Thr Gly 545 550 560 Trp His Ala Lys Cys Leu Asp Phe Phe Lys Ser Arg Glu Gly Arg Phe 565 570 575 Ala Phe Val Thr Thr Asn Ser Ile Thr Gln Gly Asp Gln Val Pro Arg 580 585 590 Leu Phe Gly Pro Ile Phe Lys Ala Gly Trp Arg Ile Arg Phe Ala His 595 600 605

NEB-207-US.ST25.txt Thr Phe Ala Trp Asp Ser Glu Ala Pro Gly Lys Ala Ala Val His 610 620 Cys Val Ile Val Gly Phe Asp Lys Glu Ser Gln Pro Arg Pro Arg Leu 625 630 635 640 Trp Asp Tyr Pro Asp Val Lys Gly Glu Pro Val Ser Val Glu Val Gly 645 650 655 Gln Ser Ile Asn Ala Tyr Leu Val Asp Gly Pro Asn Val Leu Val Asp 660 665 670 Lys Ser Arg His Pro Ile Ser Ser Glu Ile Ser Pro Ala Thr Phe Gly 675 680 685 Asn Met Ala Arg Asp Gly Gly Asn Leu Leu Val Glu Val Asp Glu Tyr 690 700 Asp Glu Val Met Ser Asp Pro Val Ala Ala Lys Tyr Val Arg Pro Phe 705 710 715 720 Arg Gly Ser Arg Glu Leu Met Asn Gly Leu Asp Arg Trp Cys Leu Trp 725 730 735 Leu Val Asp Val Ala Pro Ser Asp Ile Ala Gln Ser Pro Val Leu Lys 740 745 750 Lys Arg Leu Glu Ala Val Lys Ser Phe Arg Ala Asp Ser Lys Ala Ala 755 760 765 Ser Thr Arg Lys Met Ala Glu Thr Pro His Leu Phe Gly Gln Arg Ser 770 775 780 Gln Pro Asp Thr Asp Tyr Leu Cys Leu Pro Lys Val Val Ser Glu Arg 785 790 795 800 Arg Ser Tyr Phe Thr Val Gln Arg Tyr Pro Ser Asn Val Ile Ala Ser 805 810 815 Asp Leu Val Phe His Ala Gln Asp Pro Asp Gly Leu Met Phe Ala Leu 820 825 830 Ala Ser Ser Ser Met Phe Ile Thr Trp Gln Lys Ser Ile Gly Gly Arg 835 840 845 Leu Lys Ser Asp Leu Arg Phe Ala Asn Thr Leu Thr Trp Asn Thr Phe 850 860

Pro Val Pro Glu Leu Asp Glu Lys Thr Arg Gln Arg Ile Ile Lys Ala 865 870 875 880

Gly Lys Lys Val Leu Asp Ala Arg Ala Leu His Pro Glu Arg Ser Leu 885 890 895

Ala Glu His Tyr Asn Pro Leu Ala Met Ala Pro Glu Leu Ile Lys Ala 900 905 910

His Asp Ala Leu Asp Arg Glu Val Asp Lys Ala Phe Gly Ala Pro Arg 915 920 925

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Glu Tyr Ile Arg Gly Glu Val Arg Phe Cys Leu Trp Ile Ser Asp Ser 50 60

Gln Glu Gln Glu Ala Lys Ser Asn Ser Asp Ile Asn Cys Lys Leu Asn 65 70 75 80

Ala Val Ala Ala Phe Arg Leu Lys Ser Pro Lys Ala Ala Thr Lys Lys 85 90 95

Gly Ala Ala Trp Pro His Lys Phe Glu Glu Val Lys Gln Ile Gly Asn 100 105 110 NEB-207-US.ST25.txt
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115 120 125

Leu Pro Val Gly Leu Leu Pro Arg Gly Ser Ile Val Thr Asp Leu Ala 130 135 140

Phe Ala Leu Tyr Asp Ala Pro Leu Trp Asn Met Ala Leu Ile Ala Ser 145 150 155 160

Arg Leu His Leu Val Trp Ile Gly 165

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Ser Asp Leu Pro Gly Ala Val Leu Gln Arg Asn His Ile His Ile Ala 50 60

Thr Cys Asp Ala Gly Asn Val Asp Arg Thr Leu Ala Ala Leu Arg Lys 65 70 75 80

Ser Pro Lys Thr Ala Ser Gln Lys Ala Arg Phe Ile Leu Ala Thr Asp 85 90 95

Gly Val Ala Phe Gln Ala Glu Asp Met Ala Ser Gly Glu Thr Val Ala 100 105 110

Cys Asn Tyr Ala Ala Phe Pro Asp Lys Phe Ala Phe Phe Leu Pro Leu 115 120 125

Ala Gly Ile Thr Thr Val Gln Gln Ile Arg Glu Ser Ser Phe Asp Ile 130 140

Lys Ala Thr Gly Arg Leu Asn Lys Leu Tyr Val Glu Leu Leu Lys Asp Page 30

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Pro Leu Thr Asn Phe Arg Gly Ile Glu Leu Arg Asn Phe Pro Ala Glu 385 390 395 400

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165 170 175 Phe Ser Ala Ile Ala Leu Arg Val Gln Gly Arg Gly Thr Pro Asp Glu

Page 34

Ile Ala His Phe Val Asn Gln Leu Val Phe Cys Phe Phe Ala Gln Ser

Val Ser Leu Leu Pro Asp Gly Leu Phe Thr Lys Leu Leu Lys Arg Ser 210 215 220 Ala Arg Ala Pro Glu Arg Ala Met Ser Tyr Leu Asp Lys Leu Phe Glu 225 230 235 240 Ala Met Glu Arg Gly Glu Phe Asp Leu Thr Asp Ile Thr Trp Phe 245 250 255 Asn Gly Gly Leu Phe Asp Gly Arg Arg Ala Leu Arg Leu Asp Asp Gly 260 265 270 Asp Ile Gly Leu Leu Val Ala Ala Asp Ser Leu Asp Trp Gly Leu Ile 275 280 285 Pro Thr Ile Phe Gly Thr Leu Phe Glu Arg Phe Leu Asp Pro Glu 290 295 300 Arg Ala Gln Ile Gly Ala His Tyr Thr Asp Pro Glu Lys Ile Met 310 315 320 Arg Leu Val Asp Pro Val Ile Leu Arg Pro Leu Arg Gln Glu Trp Glu 325 330 335 Gln Ala Arg Arg Glu Ile Val Glu Leu Leu Asn Gly Asn Arg Lys Pro 340 345 350 Pro Met Arg Arg Gln Gln Ser Arg Arg Met Lys Arg Glu Glu Ala Ala 355 360 365 Ala Glu Val Arg Ser Arg Phe Thr Glu Arg Leu Arg Lys Leu Arg Ile 370 375 380 Leu Asp Pro Ala Cys Gly Ser Gly Asn Phe Leu Tyr Leu Ala Leu Gln 385 390 395 400 Gly Val Lys Asp Ile Glu His Arg Ala Asn Leu Asp Cys Glu Met Leu 405 410 415 Gly Met Pro Ala Gln Leu Pro Leu Val Gly Pro Glu Ile Leu Arg Gly 420 425 430 Ile Glu Ile Asn Met Met Ala Ala Glu Leu Ala Arg Thr Thr Ile Trp 435 440 445 Ile Gly Asp Ile Gln Trp Gln Ile Lys Asn Gly Ile Arg Ser Lys Ser
450 455 460 Page 35

Ile Pro Ile Leu Arg Lys Leu Asp Ala Ile Glu Arg Arg Asp Ala Leu 465 470 475 480 Val Arg Gln Ala Gln Asp Val Asp Thr Ala Arg Asp Ala Gln Gly Asp 485 490 495 Leu Leu Ala Ala Leu Gln Pro Val Ser Glu Asp Ala Glu Ala Glu Trp 500 505 510 Pro Glu Ala Glu Phe Ile Val Gly Asn Pro Pro Phe Val Gly Val Arg 515 520 525 Leu Met Arg Gln Ala Leu Gly Asp Pro Thr Val Asp Arg Leu Phe Asp 530 540 Val Tyr Asp Gly Arg Val Ser Arg Glu Ala Asp Leu Val Cys Tyr Trp 545 550 555 560 Val Glu Lys Ser Arg Ala Ala Val Ala Ala Asp Arg Thr Arg Arg Val 565 570 575 Gly Leu Val Thr Thr Asn Ser Ile Arg Gly Gly Ala Asn Arg Arg Val 580 585 590 Leu Asp Arg Ile Ile Ala Glu Ser Arg Leu Phe Glu Ala Trp Ser Asp 595 600 605 Glu Pro Trp Val Val Asp Gly Ala Ala Val Arg Val Ser Leu Ile Cys 610 620 Phe Gly His Gly Glu Asp Pro Leu Cys Leu Asp Gly Arg Thr Val Ala 625 635 640 Gln Ile Asn Ala Asp Leu Thr Ala Gly Val Thr Asp Leu Thr Lys Ala 645 650 655 Arg Arg Leu Ser Glu Asn Gln Asn Val Ala Phe Met Gly Asp Thr Lys 660 670 Gly Gly Ala Phe Asp Val Pro Gly Ser Leu Ala Arg Ala Trp Leu Ser 675 680 685 Met Pro Met Asn Pro Asn Gly Arg Pro Asn Ser Asp Val Leu Arg Pro 690 700 Trp Arg Asn Gly Met Asp Val Ala Arg Arg Gly Arg Asp Met Trp Ile

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Lys Leu Pro Ile Ala Gly Gly Arg Lys Ser Val Val Gly Pro Gln 1010 1015 1020

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Ala Tyr Ala Leu Asp Leu Gln Asn Pro Pro Tyr Leu Val Val Ser Asp 65 70 75 80

Met Glu Arg Ile Ile Val His Thr Asn Trp Thr Asn Thr Ile Ser Arg 85 90 95

Lys Ile Glu Phe Thr Leu Asp Asp Leu His Glu Pro Glu Lys Leu Ala 100 105 110

Met Leu Arg Gln Val Phe Asp Gly Ser Asp Ser Leu Lys Pro Lys Ile Page 38

Pro Gln Glu Leu Thr Ala Lys Val Ala Gln Arg Phe Gly Asp Leu 130 135 140 Gly Arg Arg Leu Gln Glu Arg Gly His His Pro Arg Asp Val Ala His 145 150 155 160 Phe Leu Asn Arg Val Val Phe Cys Met Phe Ala Glu Asp Ala Lys Leu 165 170 175 Leu Pro Glu Gly Leu Phe Thr Arg Leu Thr Arg Ser Met Gln Met Arg 180 185 190 Pro Ala Glu Ala Ala Pro Gln Phe Asp Ala Leu Phe Ala Met Met 195 200 205 Ala Gly Gly Met Phe Gly Ala Asp Ile Val His Trp Phe Asn Gly 210 220 Gly Leu Phe Asp Glu Lys Pro Ala Leu Pro Leu Glu Arg Ala Asp Ile 225 230 235 240 Lys Leu Ile His Asp Thr Ala Ala Glu His Asp Trp Ser Asp Leu Asp 245 250 255 Pro Ser Val Phe Gly Asn Met Phe Glu Glu Ala Leu Lys Ala Thr Arg 260 265 270 Glu Arg Ala Ala Leu Gly Ala His Tyr Thr Asp Arg Glu Lys Ile Leu 275 280 285 Lys Ile Ile Asp Pro Val Ile Thr Trp Pro Leu Met Ala Gln Trp Glu 290 295 300 Thr Ala Leu Ala Glu Ile Arg Ala Ala Leu Asp Ala Arg Ala Ala 305 310 315 320 Glu Ala Glu Arg Lys Ala Val Leu Glu Ala Ala Glu Ala Met Arg 325 330 335 Ala Asp Pro Val Lys Ala Lys Ala Gly Glu Ala Ala Arg Arg Lys Thr 340 345 350

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Arg Arg Val Thr Lys Val Gly Thr Lys Asp Lys Gly Phe Ala Asp Val 50 60

Phe Lys Lys Ala His Phe Ile Thr Glu Tyr Lys Arg Pro Gly Ser Asp 65 70 75 80

Leu Gly Ala Ala Leu Gln Gln Ala Thr Leu Tyr Ser Arg Asp Leu Gly 85 90 95

Asn Pro Pro Leu Leu Leu Thr Ser Asp Phe Gln Arg Ile Glu Ile Asn 100 105 110

Thr Ala Phe Thr Gly Thr Ser Pro Lys Ser Tyr Leu Ile Thr Leu Asp 115 120 125

Asp Ile Ala Glu Asn Arg Val Val Gly Gly Asn Asp Val Pro Ala Leu 130 135 140 Gln Ile Leu His Ser Ala Leu His Gln Pro Tyr Asp Leu Asp Pro Arg 145 150 155 160 Leu Phe Arg Glu Arg Ile Thr Thr Asp Ala Thr Arg Gln Val Gly Leu 165 170 175 Val Ala Arg Arg Leu Gly Glu Arg Glu Gly Arg Thr Arg Ala Ala His 180 185 190 Met Met Met Arg Val Val Phe Ala Leu Phe Ala Glu Asp Thr Gly Met 195 200 205 Leu Glu Arg Gly Ile Val Thr Arg Leu Leu Glu Arg Ala Arg Ala Pro 210 215 220 Pro Gly Glu Asp Gln Leu Tyr Phe Gln Asp Leu Phe Gly Ala Met Lys 235 230 240 Gly Gly Glu Phe Trp Gly Thr Asp Ile Arg His Phe Asn Gly Gly 245 250 255 Leu Phe Asp Ser Glu Asp Ala Leu Ala Leu Thr Ser Glu Asp Ala Ala 260 265 270 Ala Leu Ile Ile Ala Ala Lys Leu Asp Trp Ser Glu Val Glu Pro Ser 275 280 285 Ile Phe Gly Thr Leu Phe Glu Asn Ser Leu Asp Val Asp Thr Arg Ser 290 295 300 Arg Arg Gly Ala His Tyr Thr Ser Val Asn Asp Ile Glu Arg Ile Val 305 310 315 Asp Arg Val Val Met Glu Pro Leu Trp Ala Glu Trp Asp Ala Leu Arg 325 330 335 Leu Ser Leu Pro Glu Leu Lys Lys Asn Val Arg Leu Glu Arg Leu Phe 340 345 350Ala Phe Gln Asp Arg Leu Thr Ala Val Arg Ile Leu Asp Pro Ala Cys 355 360 365 Gly Ser Gly Asn Phe Leu Phe Val Ala Leu Lys Lys Leu Leu Asp Leu 370 380 Page 43

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Leu Arg Thr Ser Pro Pro His Pro Thr Arg Tyr Val Ile Asp Phe Gln 680 Arg Asp Val Phe Gly Ala Arg Ala Tyr Lys Leu Pro Phe Ala Arg 690 695 700 Ile Glu Arg Glu Val Leu Pro Thr Arg Gln Ala Ala Ala Ala Glu Glu 705 710 715 720 Glu Ala Arg Asn Ala Glu Val Leu Ala Ala Asn Pro Lys Ala Lys Thr 725 730 735 Asn Lys His His Arg Asn Phe Leu Asn Gln Trp Trp Ala Leu Ser Tyr 740 745 750 Gly Arg Ser Glu Met Ile Glu Lys Ile Ser Ser Leu Ser Arg Tyr Ile 755 760 765 Val Cys Ser Arg Val Thr Lys Arg Gln Val Phe Glu Phe Leu Asp Asn 770 775 780 Gly Ile Arg Pro Ser Asp Gly Leu Gln Ile Phe Ala Phe Glu Asp Asp 785 790 795 800 Tyr Ser Phe Gly Val Ile Gln Ser Ser Val His Trp Gln Trp Leu Ile 805 810 815 Ala Arg Gly Gly Thr Leu Thr Ala Arg Leu Met Tyr Thr Ser Asp Thr 820 825 830 Val Phe Asp Thr Phe Pro Trp Pro Asp Pro Thr Leu Ala Gln Val Arg 835 840 845 Ala <u>Val</u> Ala Ala Ala Val Lys Leu Arg Glu Leu Arg Asn Lys Val Met Arg Glu Gln Gly Trp Ser Leu Arg Asp Leu Tyr Arg Thr Leu Asp 865 870 880 Met Pro Gly Lys Asn Pro Leu Arg Asp Ala Gln Glu Arg Leu Asp Ala 885 890 895 Ala Val Ser Ala Ala Tyr Gly Leu Pro Ala Gly Ala Asp Met Leu Asp 905

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930 935 940
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       R = A \text{ or } G
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<221> misc_feature
<222> (15)..(15)
```

. .

. 6 1 4

```
<223> R = A or G
<400> 23
atggaygcna araarcg
                                                                                                    17
<210> 24
<211> 17
<212> DNA
<213> unknown
<220>
<223> reverse primer
<220>
<221> misc_feature <222> (6)..(6)
\langle 223 \rangle Y = T or C
<220>
<221> misc_feature
<222> (9)..(9)
<223> n is a, c, g, or t
<220>
<221> misc_feature
<222> (12)..(12)
\langle 223 \rangle R = A or G
<220>
<221> misc_feature
<222> (15)..(15)
<223> R = A or G
<400> 24
atggaygcna araarag
                                                                                                    17
<210>
        20
<211>
<212> DNA
<213> unknown
<220>
<223> reverse primer
<220>
<221> misc_feature
<222> (3)..(3)
<223> n is a, c, g, or t
<220>
<221> misc_feature
<222> (6)..(6)
<223> Y = T or C
<220>
<221> misc_feature
<222> (9)..(9)
<223> Y = T or C
```

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<220>

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<220>
<221> misc_feature
<222> (12)..(12)
<223> n is a, c, g, or t
<220>
<221> misc_feature <222> (15)..(15)
<223> R = A or G
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cgncgyttyt tngcrtccat
                                                                                       20
<210> 26
<211> 7
<212> PRT
<213> unknown
<223> 7.5 kD fragment primer
<400> 26
Asp Lys Ala Tyr Asp Gln Ala
1 5
<210> 27
<211>
       20
<212> DNA
<213> unknown
<220>
<223> forward primer
<220>
<221> misc_feature <222> (3)..(3)
        (3)..(3)
\langle 223 \rangle Y = T or C
<220>
<221> misc_feature
<222> (6)..(6)
<223> R = A or G
<220>
       misc_feature
<221>
<222> (9)..(9)
<223> n is a, c, g, or t
<220>
<221> misc_feature
<222> (11)..(11)
       (11)..(11)
<223> Y = T or C
<220>
<221> misc_feature <222> (14)..(14)
<223> Y = T or C
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. . . .

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<221> misc_feature
<222> (17)..(17)
<223> R = A or G
<400> 27
                                                                                                20
gayaargcnt aygaycargc
<210> 28
         20
<211>
<212> DNA
<213> unknown
<220>
<223> reverse primer
<220>
<221> misc_feature
<222> (3)..(3)
\langle 223 \rangle Y = T or C
<220>
<221> misc_feature
<222> (6)..(6)
<223> R = A or G
<220>
<221> misc_feature
<222> (9)..(9)
<223> R = A or G
<220>
<221> misc_feature
<222> (12)..(12)
<223> n is a, c, g, or t
<220>
<221> misc_feature
<222> (15)..(15)
<223> Y = T or C
<220>
<221> misc_feature <222> (18)..(18)
<223> R = A or G
<400> 28
gcytgrtcrt angcyttrtc
                                                                                                20
<210> 29
<211> 26
<212> DNA
<213> unknown
<220>
<223> primer IP 1
<400> 29
gttggatccc gcacagattg ctcagg
                                                                                                26
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NEB-207-US.ST25.txt <210> 30 <211> 30 <212> DNA <213> unknown <220> <223> primer IP 2 <400> 30 gttggatcct acgttaatct gaataagatg 30 <210> 31 28 <211> <212> DNA <213> unknown <220> <223> primer IP 3 <400> 31 28 gttggatcct gttaatctga aacgctgg <210> 32 <211> 29 <212> DNA <213> unknown <220> <223> primer IP 4 <400> 32 gttggatcct tataccaaaa tgtgaggtc 29 <210> 33 <211> 20 <212> DNA <213> unknown <220> <223> primer IP 5 <400> 33 ttcagaaata cgagcgatgc 20 <210> 34 <211> 20 <212> DNA <213> unknown <220> <223> primer IP 6 <400> 34 gtcaagccat aaacaccatc 20 <210> 35 <211> 20 <212> DNA

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<213>	unknown	NEB-207-US.ST25.txt	
<220> <223>	primer IP 7		
<400> gagggt	35 caga aaggaagctg	2	0
<210> <211> <212> <213>			
<220> <223>	primer IP 8		
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<210> <211> <212> <213>	20		
<220> <223>	primer IP 9		
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<210> <211> <212> <213>	20		
<220> <223>	primer IP 10		
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<210> <211> <212> <213>	39 20 DNA unknown		
<220> <223>	primer IP 11		
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<210> <211> <212> <213>	40 20 DNA unknown		
<220>		50	

<223>	primer IP 12	
	40 gcgc cctgaattag	20
<210> <211> <212> <213>	DNA	
<220> <223>	primer S1	
<400> gcttca	41 tttc atcctctgtg c	21
<210> <211> <212> <213>	42 21 DNA unknown	
<220> <223>	primer S2	
<400> taaccg	42 ccaa aattaatcgt g	21
<210> <211> <212> <213>	43 20 DNA unknown	
<220> <223>	primer S3	
<400> ccacta	43 ttca ttacaacacc	20
<210> <211> <212> <213>	44 43 DNA unknown	
<220> <223>	20 nucleotides that matched the M. methyltrophus DNA sequence	
<400> gttctg	44 cagt taaggataac atatggcttt aagctggaac gag	43
<210> <211> <212> <213>	45 37 DNA unknown	
<220> <223>	22 nucleotides that matched the M. methylotrophus DNA sequence	
<400>	45	

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NEB-207-US.ST25.txt
gttggatccg tcgacattaa ttaatttttg cccttag
                                                                        37
<210>
       46
<211>
       48
<212>
       DNA
<213>
       unknown
<220>
<223> oligonucleotide 1
<400> 46
                                                                        48
gtttgaagac tccgacgcga tggccagcga tcggcgcctc agcttttg
<210>
       47
<211>
      48
<212>
      DNA
<213>
      unknown
<220>
<223> oligonucleotide 2
<400> 47
caaaagctga ggcgccgatc gctggccatc gcgtcggagt cttcaaac
                                                                        48
<210>
       48
<211>
       48
<212>
      DNA
<213> unknown
<220>
<223> oligonucleotide 3
<220>
<221> misc_feature
<222>
      (15)..(15)
<223>
      A = 6-methyladenine
<400>
gtttgaagac tccgacgcga tggccagcga tcggcgcctc agcttttg
                                                                        48
<210>
       49
<211>
      48
<212>
      DNA
<213>
      unknown
<220>
<223> oligonucleotide 4
<220>
<221>
      misc_feature
      (38)..(38)
<222>
     A = 6-methyladenine
<223>
<400> 49
caaaagctga ggcgccgatc gctggccatc gcgtcggagt cttcaaac
                                                                        48
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. . . .

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<210> 50
<211> 8
<212> PRT
<213> unknown
<220>
<223> single internal CnBr digestion fragment
<400> 50

Gly Arg Gly Arg Gly Val Gly Val
1 5
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